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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: William S. Caldwell et al.

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Serial No.: 09/522,117

Examiner: V. Balasubramanian

Filed: March 9, 2000

For: COMPOUNDS CAPABLE OF ACTIVATING CHOLINERGIC RECEPTORS

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**DECLARATION UNDER 37 CFR §1.132**

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I, William S. Caldwell, do hereby declare and say as follows:

1. I am a co-inventor on the above-referenced patent application and am familiar with the contents thereof. I have reviewed the final Official Action mailed May 21, 2001 and am familiar with the contents thereof. I have also reviewed U.S. Patent No. 5,861,423 to Caldwell et al. (hereinafter Caldwell), which is cited in the final Office Action.

2. I received my B.S. in chemistry from The University of the South in 1976. I received my Ph.D. in chemistry from The University of Wisconsin in 1986, where I concentrated on Organic Chemistry and Enzymology. From 1985 to 1987 I was a post-doctoral researcher in the laboratory of Dr. Mark Jaffe, a professor at Wake Forest University, where I conducted research in Plant Physiology (chemical correlates of floral induction). From 1987 to 1998 I was a researcher and research manager at R.J. Reynolds Tobacco where my technical duties included research in physical organic chemistry, mechanistic toxicology, xenobiotic metabolism, pharmacology and medicinal chemistry. I have published over 80 papers, book chapters and abstracts and am an inventor on over 40 pharmaceutical-related patents and patent applications. Since 1999, I have been Senior Manager, Director and Vice President for Drug Discovery at Targacept, Inc., where I am responsible for directing all activities in the Drug Discovery Department including Molecular Design, Medicinal Chemistry, Analytical Chemistry/QA, Pharmaceuticals and Process R&D. I also hold adjunct faculty appointments in the Chemistry Department of Wake Forest University and in the Physiology/Pharmacology Department at Wake Forest University School of Medicine.

4. I am also a co-inventor on the Caldwell reference that is cited in the final Office Action. This reference describes pharmaceutical compounds having a pyridine ring coupled to an amino group by an unsubstituted alkylene bridging moiety. I was involved in the development and evaluation of the compounds of Caldwell. While these compounds originally looked promising in *in vitro* tests, such as the tests described in the Examples of Caldwell, we found through *in vivo* testing that compounds having unsubstituted alkylene bridging moieties such as those proposed in Caldwell were readily metabolized, and attained only relatively low circulating plasma levels in test animals. As a result, such compounds did not possess an optimum pharmacokinetic profile.

We were interested in discovering compounds that (1) possessed good binding and functional characteristics (*e.g.*, characteristics comparable to those possessed by compounds having unsubstituted alkylene bridging moieties, such as those of Caldwell) and (2) possessed good pharmacokinetic profiles (*e.g.*, were not readily metabolized in the body).

5. In general, the binding characteristics of a particular compound may be evaluated by determining an inhibition constant ( $K_i$  value) for the compound.  $K_i$  values are reported in units of concentration (nM).  $K_i$  values may be calculated from  $IC_{50}$  values as described in the present specification at pages 29-31.  $IC_{50}$  values are estimated as the concentration of compound that inhibited 50 percent of specific L-[ $^3H$ ]nicotine binding. Thus, better binding is evidenced by a lower  $K_i$  value, which indicates that a lower concentration of compound was needed to inhibit specific L-[ $^3H$ ]nicotine binding. In general, utilizing the disclosed method of determining  $K_i$  values results in  $K_i$  values that are accurate to within approximately a factor of 2. I understand that during the prosecution of this application, our attorneys may have indicated that higher  $K_i$  values are preferable and/or that  $K_i$  values possess greater accuracy than that just described. In order to clarify any possible misunderstanding, these assertions are not technically correct. It is my understanding that they were not made with an intent to deceive the Patent Office, however.

In general, pharmacokinetic profile characteristics of a particular compound may be evaluated by administering the compound to a subject and determining the plasma levels of the compound over a given time. Two useful measures of a pharmacokinetic profile are

Cp<sub>max</sub>, which is an estimate of the maximum plasma level of the compound, and AUC (area under the plot of plasma concentration of drug against time after drug administration), which is useful in estimating the bioavailability of the compound. For more preferred pharmacokinetic profiles, the Cp<sub>max</sub> and AUC will generally be higher.

6. In an effort to provide a compound that possessed both good binding/functional characteristics and good pharmacokinetic profiles, we initially attempted to vary the substituent at the 5-position of the pyridine ring of the N-methyl-4-(3-pyridinyl)-3-buten-1 amine compound described in Caldwell. As illustrated by the data in Table 1 at Appendix A, varying the substituent at the 5-position of the pyridine ring resulted in compounds that retained binding at  $\alpha 4\beta 2$  receptor as evidenced by the Ki value. However, such variations at the 5-position did not solve the metabolism problem. All compounds in Table 1 exhibit relatively low circulating levels as evidenced by the Cp<sub>max</sub> and AUC values. These results, and the isolation of metabolites, indicated to us that the problem might be monoamine oxidase activity at the secondary amine side chain.

7. We then tried various substitutions at other positions on the N-methyl-4-(3-pyridinyl)-3-buten-1 amine compound described by Caldwell. In one compound, we added a single methyl group at the 2-position of the pyridine ring. In another compound, we added a single methyl group at the 6-position of the pyridine ring. In still another compound, we added a single methyl group to the amino group, thus forming a tertiary amine. In yet another compound, we changed the N-methyl group to an N-isopropyl group. As illustrated by the Ki values for the structures shown in Table 2 at Appendix B, we found that these substitutions resulted in dramatically reduced binding characteristics.

8. After the various trials detailed in paragraphs 6 and 7 above, we were inspired to consider another pharmacological field, that of  $\beta$ -phenethylamine and amphetamine chemistry. We reviewed various references describing the pharmacokinetics of phenethylamine and amphetamine chemistry including Shannon, et al., "Physiologic Effects and Plasma Kinetics of  $\beta$ -Phenylethylamine and Its N-Methyl Homolog in the Dog", *J.*

*Pharmacol. Exp. Ther.*, **223**(1): 190-196 (1982); Baggot, et al., "Comparative Study of the Pharmacokinetics of Amphetamine", *Res. Vet. Sci.*, **14**(2): 207-215 (1973); Baggot, et al., "Pharmacokinetic Study of Amphetamine Elimination in Dogs and Swine", *Biochem. Pharmacol.*, **21**(14): 1967-1976 (1972); and Edgar, et al., "Pharmacokinetics of Methamphetamine Self-administered to Human Subjects by Smoking S-(+)-Methamphetamine Hydrochloride", **21**(4): 717-723 (1993). In our review of the literature, we found that installation of a methyl group  $\alpha$  to (*i.e.*, on the carbon attached to) the amine nitrogen was known to retard the action of monoamine oxidase for  $\beta$ -phenethylamine and amphetamine compounds, and thus prolong the plasma half-life of these compounds (*see*, Table 3 at Appendix C).

Although Applicants do not wish to be bound by a single theory, the probable explanation of this effect is that the  $\alpha$ -methyl group creates a degree of steric hindrance, relative to the unsubstituted material, which hinders binding at the oxidase active site.

9. We believed that the addition of a methyl, or other alkyl group, alpha to the amino group in the compounds of Caldwell could result in a  $-\text{CH}(\text{CH}_3)\text{NH}(\text{CH}_3)$  amino structure similar to the amino structure of  $\alpha$ -methyl versions of  $\beta$ -phenethylamines (*i.e.*, amphetamines). If the poor metabolic characteristics of the compounds of Caldwell were, in fact, due to monoamine activity at the secondary amine side chain, we hoped that employing a structure similar to the amino structure of  $\alpha$ -methyl versions of  $\beta$ -phenethylamines might result in the desired improvement in metabolic characteristics.

However, we realized that various structural differences exist between metanicotine compounds, such as those described in Caldwell, and amphetamine compounds. For example, metanicotines have a pyridinyl ring while amphetamines have a phenyl ring. Also, metanicotines have an alkenyl bridging moiety while amphetamines have an alkyl bridging moiety. Given these structural differences, we were not certain that employing an amino structure on the metanicotine compound similar to the amino structure of  $\alpha$ -methyl versions of  $\beta$ -phenethylamines would decrease the monoamine activity at the secondary amine side chain and result in the desired improvement in metabolic characteristics.

Even if such an  $\alpha$ -methyl amino structure did result in decreased monoamine activity at the secondary amine side chain of the metan nicotine compound, we were concerned that this  $\alpha$ -methyl structure could result in an undesirable and/or unacceptable reduction in binding at the  $\alpha 4\beta 2$  receptor. As described above in paragraph 8, we believed that the improved metabolic characteristics observed in the amphetamine art might be due to the  $\alpha$ -methyl group creating a degree of steric hindrance, relative to the unsubstituted material, which may hinder binding at the oxidase active site. We were concerned that the degree of steric hindrance that resulted in hindered binding at the oxidase active site providing improved metabolic characteristics might also hinder binding at the  $\alpha 4\beta 2$  receptor resulting in an undesirable and/or unacceptable reduction in activity at the  $\alpha 4\beta 2$  receptor.

These concerns were heightened in view of our experience with the methyl substitutions described above in paragraph 7. Recall, for example, that addition of a methyl group to the amino group had resulted in a drastic decrease in binding at the  $\alpha 4\beta 2$  receptor.

Moreover, as summarized in Table 4 at Appendix D, our own experience with the effect of substituents on the binding of (S)-(-)-nicotine at the  $\alpha 4\beta 2$  receptor indicated that addition of a methyl group alpha to the amino group may result in an unacceptable decrease in binding. For example, we had previously determined that substituting a methyl group alpha to the cationic site in (S)-(-)-nicotine (Compound 5 in Table 4) resulted in a  $K_i$  value of 6400 nM, which was drastically worse than the  $K_i$  value of 2 for (S)-(-)-nicotine (Compound 1 in Table 4).

Furthermore, the experience of other researchers in the field indicated that the addition of a methyl group alpha to the cationic site in (S)-(-)-nicotine could result in decreased binding at the  $\alpha 4\beta 2$  receptor. For example, Lin, et al., "Synthesis and Evaluation of Nicotine Analogs as Neuronal Nicotine Acetylcholine Receptor Ligands", *J. Med. Chem.*, **37**: 3542-3553 (1994), provided at Appendix E, gives  $K_i$  values for  $\alpha$ -methyl substituted (S)-(-)-nicotine that are a factor of about 30 to over 1000 higher than the  $K_i$  value for (S)-(-)-nicotine (see, Lin, et al., page 3545, table 1, compounds 35 and 36 compared with compound (S)-nicotine). Based on our own experience and the experience of other researchers in the field, we thought it likely that the same steric factors responsible for decreased binding at the oxidase might result in decreased binding to the nicotinic receptor.

10. We were also inspired to consider the effect of the stereochemistry of the  $\alpha$ -methyl group. By reviewing the literature, we discovered that for various other classes of nicotinic compounds (including nicotine itself), stereochemistry at the site of substitution may be an important determinant of nicotinic binding. For example, the Lin article, discussed above in paragraph 9 and provided at Appendix E, reported that the (S)-nicotine compound having an  $\alpha$ -methyl group of alpha stereochemistry (*cis* to the pyridine ring) had a  $K_i$  value of about 1205 nM, while the (S)-nicotine compound having an  $\alpha$ -methyl group of beta stereochemistry (*trans* to the pyridine ring) had a  $K_i$  value of about 35. Thus, while the Lin article had unfavorably showed that the  $\alpha$ -methyl group may inhibit binding at the  $\alpha 4\beta 2$  receptor, it appeared to show that perhaps one of the two enantiomers of the  $\alpha$ -methyl compounds according to embodiments of the present invention might possess binding characteristics that were better than the binding characteristics of the other enantiomer. Such a conclusion was by no means a certainty, however, given the difference in structure between the (S)-nicotine compound and the metanicotine compounds.

Our review of the literature regarding the stereochemical effects of substituents on acyclic amines such as metanicotine revealed WO 96/08468 to Falch, provided at Appendix I, which may be the most relevant art in this area. Falch reports  $IC_{50}$  values for two sets of enantiomers having a methyl group alpha to the amino moiety of an acyclic cationic side chain. As described above in paragraph 5,  $IC_{50}$  values are estimated as the concentration of compound that inhibited 50 percent of specific L-[ $^3H$ ]nicotine binding. Thus, better binding is evidenced by a lower  $IC_{50}$  value, which indicates that a lower concentration of compound was needed to inhibit specific L-[ $^3H$ ]nicotine binding. As shown in Table 2 on Page 16 of Falch, compound (S)-8 has an  $IC_{50}$  value of 0.064  $\mu M$  while compound (R)-8 has an  $IC_{50}$  value of 0.003  $\mu M$  and compound (S)-30 has an  $IC_{50}$  value of 0.39  $\mu M$  while compound (R)-30 has an  $IC_{50}$  value of 0.006  $\mu M$ . Falch shows that the R enantiomer of a compound having a methyl group alpha to the amino moiety of an acyclic cationic side chain possesses better binding characteristics than the S enantiomer of the compound.

11. Given the uncertainties and concerns regarding  $\alpha$ -methyl substitution described above in paragraph 9, we were surprised to find that the compounds according to

embodiments of the present invention showed improved metabolic characteristics and retained binding at the  $\alpha 4\beta 2$  receptor. As illustrated in Table 5 at Appendix G, compounds according to embodiments of the present invention (Compounds 2 and 3) possesses improved metabolic characteristics when compared with the prior art compounds of Caldwell (Compound 1) as illustrated by their  $C_p$  max and AUC values, which are consistently and significantly higher than for the prior art unsubstituted compounds. This improvement in metabolic characteristics was accomplished while retaining acceptable binding at the  $\alpha 4\beta 2$  receptor as illustrated by the  $K_i$  values in Table 5, which are accurate to approximately a factor of 2.

Given the teachings of Falch, we were also surprised to discover that the S isomers invariably bind with higher affinity (lower  $K_i$ ) than the R isomers. As illustrated in Table 5, the S isomer of the 5-isopropoxy substituted alpha-methylmetanicotine (Compound 3) has a  $K_i$  value of 11 nM compared with a  $K_i$  value of 62 nM for the R isomer (Compound 2). The  $K_i$  value for Compound 3 is comparable to the  $K_i$  value for the corresponding non- $\alpha$ -methyl compound (Compound 1). Moreover, the functional activity of the S isomers at the  $\alpha 4\beta 2$  receptor was not only superior to that of the R isomers, but also superior to that of the unsubstituted analogs (see "activity ratio" in Tables 5).

12. Thus, we unexpectedly discovered that the  $\alpha$ -methyl compounds according to embodiments of the present invention typically possessed an improved overall combination of biological and pharmacokinetic characteristics (high affinity for the receptor, ability to elicit functional response at the receptor and resistance to metabolic clearance). We more particularly found that the S enantiomers of the  $\alpha$ -methyl compounds according to embodiments of the present invention resulted in the best overall combination of biological and pharmacokinetic characteristics. These characteristics make the  $\alpha$ -methyl compounds according to embodiments of the present invention significantly better drug candidates than the unsubstituted analogs.

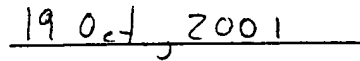
13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that

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these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



William S. Caldwell

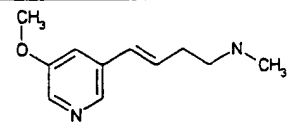
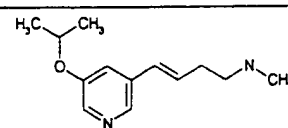
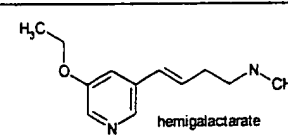
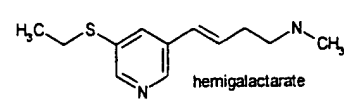


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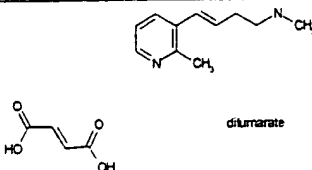
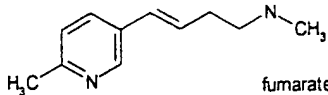
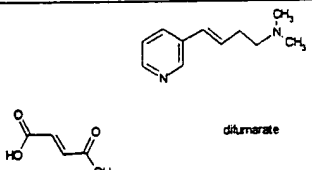
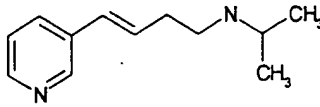


**Table 1**

Compound	STRUCTURE	Ki	Cp max (ng/mL)	AUC 0-∞ (h.ng/mL)
1		9	18	23
2		5	19	30
3		5	8	12
4		28	21	24



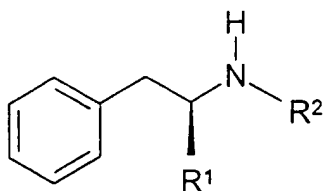
**Table 2**

Compound	STRUCTURE	Ki
1	 <chem>Cc1cc(C=CCN(C)C)ccn1</chem> <chem>OC(=O)/C=C/C(=O)O</chem> difumarate	5585
2	 <chem>Cc1cc(C=CCN(C)C)ccn1</chem> <chem>OC(=O)/C=C/C(=O)O</chem> fumarate	598
3	 <chem>C=CCN(C)Cc1ccncc1</chem> <chem>OC(=O)/C=C/C(=O)O</chem> difumarate	2067
4	 <chem>CC(C)N(C=CC1=CC=CC=C1N1)C1=CC=CC=C1</chem>	270000

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**Table 3**

**Improved Plasma Half-life for  $\beta$ -Phenethylamine Compounds  
Having an  $\alpha$ -Methyl Group**



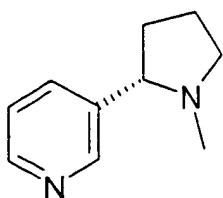
Species (route)	$R^1$	$R^2$	$t_{1/2}$
Dog (i.v.)	H	H	5-10 min
Dog (i.v.)	H	$CH_3$	5-10 min
Dog (i.v.)	$CH_3$	H	4.5 h
Human (i.v.)	$CH_3$	$CH_3$	12.2 h
Human (p.o.)	$CH_3$	$CH_3$	10.1 h



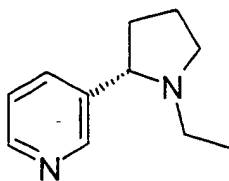
**Table 4**

**Effects of Methyl Group Substitution of (S)-(-)-Nicotine  
on the  $\alpha 4\beta 2$  Nicotinic Pharmacophore**

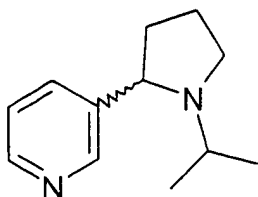
- Methyl group  $\alpha$  to N in (S)-(-)-nicotine



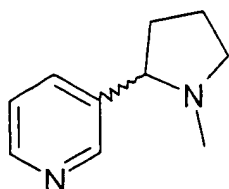
**K<sub>i</sub> = 2 nM**



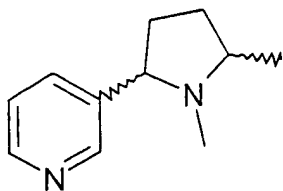
**K<sub>i</sub> = 52 nM**



**K<sub>i</sub> = 1500 nM**



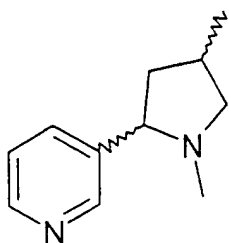
**K<sub>i</sub> = 43 nM (Literature value from M.B.)**



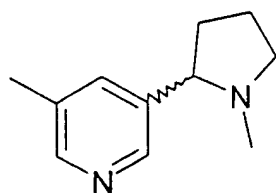
**K<sub>i</sub> = 6400 nM**



- Methyl groups not  $\alpha$  to N in (S)-(-)-nicotine



$K_i = 91 \text{ nM}$



$K_i = 2 \text{ nM}$

